REMARKS

Claims 22-26 are presented for examination. Applicants thank the Examiner for his review of the instant application. For the reasons stated below, the rejections of the presently pending claims are respectfully traversed.

Rejection Under 35 U.S.C. §101 – Utility

The PTO maintains its rejection of pending Claims 22-26 under 35 U.S.C. § 101 as lacking utility. The PTO states that while Applicants assert that "the PRO 539 protein shares the utility asserted for the PRO 539 nucleic acid as a diagnostic, that assertion is not supported by specific evidence of utility for PRO539 itself as being overexpressed in any cancer cell. The utility assertion for the PRO539 protein is based on a presumed generic relationship between mRNA expression and protein expression that is argued to exist generically. The weight of prior art which analyzes large scale sets of proteins does not support this utility, as discussed next." *Office Action* at 3.

For the reasons set forth below, Applicants respectfully disagree.

Applicants incorporate by reference their previously submitted arguments, and for the reasons of record assert that the specification contains a disclosure of utility which corresponds in scope to the subject matter sought to be patented and therefore must be taken as sufficient to satisfy the utility requirement of 35 U.S.C. § 101. Applicants also submit that for reasons of record, the PTO has not met its burden of providing evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility. However even if the PTO has met its initial burden, Applicants' rebuttal evidence previously submitted and additional evidence submitted herewith is sufficient to prove that it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability,** that the asserted utility is true.

As stated previously, Applicants' evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. Even if the correlation between Applicants evidence and the asserted utility is not exact, or a "necessary" relationship, such that there are exceptions to the correlation between the evidence and the asserted utility, this is sufficient to establish a utility. *See Fujikawa v. Wattanasin*, 93 F.3d 1559, 39 U.S.P.Q.2d 1895 (Fed. Cir. 1996) (stating that "a 'rigorous correlation' need not be shown in order to

establish practical utility; 'reasonable correlation' suffices," and thus a utility was established even though there were exceptions to the correlation between the disclosed *in vitro* data and asserted *in vivo* utility). Therefore, exceptions between the evidence disclosed and the asserted utility is permissible – **the standard is not absolute certainty or a "necessary" relationship.**

Substantial Utility

Summary of Applicants' Arguments and the PTO's Response

Applicants' asserted utility rests on the following argument:

1. Applicants have provided reliable evidence that gene for the PRO539 polypeptide is amplified by at least two-fold in lung and colon tumors (as explained previously, in Example 16 of the specification, a ΔCt value of 1.0 equals a two-fold amplification, a ΔCt value of 2.0 equals a four-fold amplification, etc.);

2. Applicants assert that it is well-established in the art that amplification of a gene leads to overexpression of the corresponding mRNA, and that a change in the level of mRNA for a particular protein, *e.g.* an increase, generally leads to a corresponding change in the level of the encoded protein, *e.g.* an increase;

3. Given the amplification of the PRO539 gene in lung and colon tumors, it is more likely than not that the PRO539 polypeptide is overexpressed in lung and colon tumors compared to their normal tissue counterparts, making the claimed antibodies useful as diagnostic tools, alone or in combination with other diagnostic tools.

Applicants understand the PTO to be making essentially two arguments in response to Applicants' asserted utility:

1. The PTO challenges the reliability and significance of the data reporting amplification of the PRO539 DNA in lung and colon tumors, making several arguments, including: (a) that the "overexpression data does not provide substantial utility" because "there is no evidence that the overexpression effect was statistically significant" or "reproducible;" *Office Action* at 8-9; (b) that "the overwhelming state of the art supports the position that many genes are irrelevant, that genes whose expression does not change are noise, and that these irrelevant genes are so insignificant that ideally they are not placed on the arrays or used at all," citing the Ding, Li, and Sawiris references for support; *id.* at 9-10; (c) that the data regarding gene amplification of

PRO539 are suspect because "tissue matched samples were not used as controls;" *id.* at 10-11; and, (d) that the data regarding gene amplification of PRO539 "do not convey utility to the claimed polypeptides, since a small amplification in genomic DNA is shown in the art to fail to correlate with a corresponding increase in mRNA and polypeptide levels," citing the Pennica, Konopka, and Gökman-Polar references for support; *id.* at 11; and

2. The PTO's second argument is that protein and DNA microarray data show no necessary correlation between mRNA overexpression and protein expression, based on the PTO's conclusions that "seven out of eight microarray papers show discordant protein and mRNA expression," and that "[a]bundant art supports the absence of a necessary relationship between mRNA and protein;" *Office Action* at 3-8.

Applicants respectfully submit that in light of all of the evidence, the PTO's arguments are not adequate to support the utility rejection of the claimed invention under 35 U.S.C. § 101.

The PTO has Acknowledged that the Data Reporting Amplification of the PRO539 Gene is Sufficient to Provide Utility for the PRO539 Nucleic Acids as a Diagnostic Tools

Applicants first address the PTO's argument that the evidence of amplification of the gene encoding the PRO539 polypeptide in lung and colon tumors is not sufficient to provide a substantial utility.

Applicants note that in the closely related application Serial No. 10/033,167, directed to nucleic acids related to SEQ ID NO:6 which encodes the PRO539 polypeptide, the PTO has acknowledged that the nucleic acids have utility. *See Notice of Allowability for Application 10/033,167 dated 7/21/2005.* In that case, the exact same data from Example 16 was relied on for utility of the claimed nucleic acids as diagnostic tools for lung and colon tumors, and the PTO made the same arguments regarding the insufficiency of the data in Example 16. *See Office Action for Application 10/033,167 dated 4/28/03* at 4. In response to Applicants' arguments to the contrary, and the Declaration of Audrey Goddard, the PTO stated "The rejection of Claims 22-41 under 35 U.S.C. § 101 is withdrawn in view of the Declaration" *See Office Action for Application 10/033,167 dated 9/9/03* at 2. Therefore, Applicants submit that the PTO's rejection of the exact same data in the instant case based on the same arguments of alleged insufficient details are moot in light of this statement. As such, the data in Example 16 are sufficient to

establish utility for the PRO539 nucleic acids as a diagnostic tool. Applicants acknowledge that an additional relationship between gene amplification and protein expression must be accepted to move from a utility for the PRO539 nucleic acids to a utility for the encoded protein and related antibodies. However, the PTO's rejection of the same gene amplification data in this application as insufficient to provide evidence that the PRO539 DNA is amplified in lung and colon tumors is arbitrary and inconsistent. The only question which remains is whether gene amplification is reasonably well correlated with protein expression such that it is more likely than not that the claimed antibodies have utility. None of the PTO's arguments attacking the sufficiency of the data in Example 16 are directed to this question, and they have already been resolved in Applicants' favor in the related application. Nonetheless, Applicants' will address each of them in turn.

In response to the specific arguments regarding the amplification data, Applicants first address the PTO's argument that the "overexpression data does not provide substantial utility" because "there is no evidence that the overexpression effect was statistically significant" or "reproducible;" *Office Action* at 8-9.

These statements are in direct contrast to the position the PTO has taken in the related nucleic acid case. In addition, as Applicants have previously stated, Applicants are not required to prove utility to a statistical certainty, only that it is more likely than not true. *See Nelson v. Bowler*, 626 F.2d 853, 856-57, 206 U.S.P.Q. 881, 883-84 (C.C.P.A. 1980) (reversing the Board and rejecting an argument that evidence of utility was insufficient because it was not statistically significant). As the M.P.E.P. states:

> [T]he applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt." **Nor must the applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty**. Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true. *M.P.E.P.* § 2107.02, part VII (bold emphasis added, underline in original, citations omitted).

Therefore, whether the results are statistically significant or not is irrelevant to establishing the asserted utility. The results must simply be reliable enough that one of skill in the art would believe that the utility is more likely than not true.

> Regarding reproducibility, the PTO states:
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> Further, there is no evidence that the overexpression was reproducible. From the data presented in the specification, a single prostate tumor sample from a single patient may have been used. Such a result from a single patient would not support any utility because even if the nucleic acid was overexpressed in the one patient, there would be no expectation that the result would appear in even one other patient, so there is no evidence of record that the overexpression shown has any utility as a diagnostic or for any other purpose. Also, there is no evidence that the over expression in the prostate tumor was anything other than a nonspecific effect due to the presence of an exogenous protein in the mixture. *Office Action* at 9, (emphasis added).

This argument is misplaced and irrelevant, as Applicants' data do not relate to prostate cancer, but rather lung and colon cancer. As described in Example 16 of the present application, gene amplification of PRO539 in a variety of primary cancers and cancer cell lines was monitored using real-time quantitative TaqMan™ PCR. The gene amplification results are set forth in Table 7 (Table 8 as amended) on page 117 of the specification. As explained in the specification on page 112, lines 17-19, the results of TaqMan™ PCR are reported in ΔCt units. It is well-known in the art that "Ct" stands for "threshold cycle." One Ct unit corresponds to one PCR cycle or approximately a 2-fold amplification, relative to control, 2 units correspond to 4-fold amplification, 3 units to 8-fold amplification, *etc.* *Specification* at 112, lines 17-19. Looking at the results reported on page 117, nine primary lung tumors and eight primary colon tumors were tested, as well as a number of tumor cell lines. PRO539 had a ΔCt value of greater than 1, *i.e.*, more than two-fold amplification, in six of nine lung tumors and five of eight colon tumors. These data show that in more than half of the lung and colon tumors tested, the gene for PRO539 was amplified at least two-fold. Therefore, the PTO's arguments regarding reproducibility are simply wrong.

The PTO also argues that "the art supports the conclusion that many genes are irrelevant in gene microarray assays." *Office Action* at 9. Relying on Li, Ding, and Sawiris, the PTO concludes that "the overwhelming state of the art supports the position that many genes are irrelevant, that genes whose expression does not change are noise, and that these irrelevant genes are so insignificant that ideally they are not placed on the arrays or used at all. The current gene, Pro9820, is such a gene." *Office Action* at 10 (emphasis added). The PTO concludes that "genes

such as Pro9820, lack substantial utility as useful on gene expression arrays." *Id.* (emphasis added).

Again, this argument is irrelevant for several reasons. First, because the instant application concerns gene amplification of PRO539 in lung and colon cancer, not Pro9820 in prostate cancer microarrays. Second, the PTO has already accepted that the PRO539 gene has utility as a diagnostic tool, as discussed above. Third, the claims of the instant application are directed to antibodies to PRO539, not genes for use on gene expression arrays.

As for the three references cited by the PTO, they do not support a rejection of the instant claims. Applicants do not dispute that many genes are irrelevant when it comes to use in gene microarrays. However, the cited references do not support the PTO's conclusion that a gene which is significantly amplified or overexpressed in certain cancer cells, such as the gene which encodes PRO539, is not useful in a gene microarray. The cited statements from Li, that there are important and irrelevant genes and that it is useful to remove the irrelevant genes from microarrays, are statements of the obvious, and offer no support for an argument that the gene encoding PRO539 is one of the irrelevant genes. To the contrary, Li goes on to analyze an example of a microarray used to distinguish cancerous tissue from normal tissue. (Li at 543.) The authors state that in making such a distinction, they are most interested in genes that are expressed higher in cancerous tissues than in normal tissues. (*Id.*) Thus, Li teaches that the gene encoding PRO539 is an example of a gene that would be of interest.

Likewise, the PTO cites Ding for the proposition that genes without changes in expression profiling should be discarded as irrelevant. Regardless of the merits of the novel method disclosed in Ding, PRO539 does show a change in expression profile between lung and colon tumors and normal tissue. Thus, nothing in Ding supports the PTO's conclusion that a gene which is significantly overexpressed in certain cancer cells, such as the gene encoding PRO539, is not useful in a gene microarray.

Finally, the PTO cites Sawiris for the obvious statement that "[o]ne of the advantages of specialized arrays is that they do not include irrelevant genes that may contribute to noise during data analysis." What the PTO fails to note is that the genes that were chosen for inclusion in the specialized chip were those that were either overexpressed or underexpressed in ovarian cancer.

(Sawiris at 2923, second column.) Thus, contrary to the PTO's assertions, the gene encoding PRO539 is useful for microarrays since it is overexpressed in certain colon and lung tumors.

Therefore, the three references cited do not support the PTO's rejection of the asserted utility of using the gene encoding PRO539 as a diagnostic agent for cancer. While the PTO's statement that the prior art supports the conclusion that there are many irrelevant genes is not disputed, none of the references support the conclusion that the gene encoding PRO539 is one of those irrelevant genes when it comes to a diagnostic tool for cancer, particularly colon and lung cancer. To the contrary, the references indicate that the relevant genes are those that are overexpressed or underexpressed in the cancer of interest, genes like the one which encodes PRO539. Thus Applicants submit that the PTO has failed to offer any support for its conclusion that the gene encoding PRO539 is not useful as a cancer diagnostic tool.

Applicants next address the PTO's argument that the data regarding gene amplification of PRO539 are suspect because "tissue matched samples were not used as controls." *Office Action* at 10-11. The PTO does not explain why tissue matched controls are necessary to evaluate gene amplification in tumor samples, as opposed to gene overexpression where tissue matched controls may be necessary. In addition, the PTO does not offer any support for its assertion that "both cancerous and non-cancerous lung tissue can be aneuploidy." *Id.* at 10. Even if it did, Applicants note that the PRO539 was also amplified in colon tumors as well.

Finally, Applicants address the PTO's argument that the data regarding gene amplification of PRO539 "do not convey utility to the claimed polypeptides, since a small amplification in genomic DNA is shown in the art to fail to correlate with a corresponding increase in mRNA and polypeptide levels," citing the Pennica, Konopka, and Gökman-Polar references for support. *Office Action* at 11.

As an initial matter, Applicants cannot find any discussion of Konopka in any previous Office Action, and therefore the PTO has failed to offer any reasoning as to how it supports the PTO's arguments.

In fact, a reading of the entire Konopka reference reveals that it does not support the PTO's argument that there is no correlation between nucleic acid levels and protein expression. Instead, the results presented in Konopka actually present strong evidence in support of

Applicants' position that there is a general understanding in the art that levels of mRNA correlate with levels of the corresponding proteins.

Konopka reports on the expression of the translocated *c-abl* oncogene, known as the Philadelphia chromosome, or Ph^1. (Konopka at 4049.) In the cancer cells studied, the Ph^1 translocation creates a chimeric *abl* gene, *bcr-abl*, that encodes a structurally altered form of the *abl* oncogene product, known as $P210^{c\text{-}abl}$. As Konopka reports, "the 8-kb mRNA that encodes $P210^{c\text{-}abl}$ was detected at 10-fold higher level in [cell type A] than in [cell type B], **which correlated with the relative level of $P210^{c\text{-}abl}$ detected in each cell line**." (*Id.* at 4050). Thus, as Applicants have asserted is most usually the case, the level of protein was correlated with the level of mRNA.

Not surprisingly, as the abstract reports, the level of protein expression of $P210^{c\text{-}abl}$ is not related to the amplification of the unaltered *abl* gene, but instead correlates to the level of mRNA for the chimeric *bcr-abl* gene, which is the product of the translocation Ph^1. Konopka thus concludes, "these combined data suggest that differential bcr-abl mRNA expression from a single gene template is responsible for the variable levels of P210c-abl [the protein of interest] detected." *Id.*, p. 4051. Thus, far from supporting the PTO's assertion that it is not the norm that increased transcription leads to increased levels of the corresponding protein, Konopka strongly supports the opposite proposition asserted by Applicants – that the level of mRNA, more often than not, correlates with the level of the corresponding protein. Thus the PTO's reliance on Konopka to support their argument that there is no correlation between nucleic acid levels and protein expression is misplaced.

Applicants have previously discussed at length why the Gökman -Polar and Pennica references do not support the PTO's position. Applicants incorporate by reference the previous arguments, including those in the Appeal Brief.

Briefly stated, Gökman-Polar reports only that increased protein levels were not accompanied by increased mRNA levels – this is not contrary to Applicants' assertion that gene amplification leads to overexpression of an mRNA which leads to overexpression of the corresponding protein. Changes in protein levels do not always and only have to be caused by changes in mRNA for Applicants' assertion to be true that changes in mRNA generally result in changes in protein. In fact, Gökman-Polar reports a positive correlation between changes in

mRNA level and changes in protein level for five of six samples tested, offering direct support for Applicants' position. Pennica, the only reference of the three which looked at a correlation between gene amplification and gene expression, reports one gene where there was a strong correlation between the two, and one possible example where there was a lack of positive correlation. This evidence is at best inconclusive, with at least half the genes showing a correlation between gene amplification and mRNA overexpression.

While these references possibly establish that there is no "necessary" correlation between gene amplification and overexpression of mRNA and protein, Applicants are not required to establish the asserted utility beyond a reasonable doubt or to a statistical certainty. Thus, even assuming that the PTO has proved that there is no "necessary" correlation, this does not mean that it has met its initial burden of establishing that it is "more likely than not" that a skilled artisan would doubt the asserted utility. Given that two of the cited references clearly support the Applicants' position, Applicants assert that the PTO has failed to meet its burden of establishing a *prima facie* case of lack of utility.

Applicants remind the PTO that Applicants enjoy a presumption that their assertions are true. The PTO must approach Applicants' assertion of utility as being sufficient to satisfy the utility requirement. M.P.E.P. §2107.02, "Procedural Considerations Related to Rejections for Lack of Utility," states:

> As a matter of Patent Office practice, a specification which contains a disclosure of utility which corresponds in scope to the subject matter sought to be patented must be taken as sufficient to satisfy the utility requirement of § 101 for the entire claimed subject matter unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope. *M.P.E.P. §2107.02* at III. A., *quoting In re Langer*, 503 F.2d 1380, 1391, 183 USPQ 288, 297 (C.C.P.A. 1974) (emphasis in original).
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> Thus, *Langer* and subsequent cases direct the Office to presume that a statement of utility made by an applicant is true. ... Office personnel should not begin by questioning the truth of the statement of utility. Instead, any inquiry must start by asking if there is any reason to question the truth of the statement of utility. ... Clearly, Office personnel should not begin an evaluation of utility by assuming that an asserted utility is likely to be false, based on the technical field of the invention or for other general reasons. *Id.*

In conclusion, Applicants submit that the evidence reported in Example 16, supported by the Goddard Declaration, establish that there is at least a two-fold amplification of the PRO539 gene in a majority of the lung and colon tumors tested. The PTO has accepted that the data in Example 16 are sufficient to establish utility for the nucleic acids encoding the PRO539 polypeptide as diagnostic tools, and therefore any challenge to the sufficiency of the data with respect to the utility of the nucleic acid is inappropriate. Therefore, the only issue which remains is whether the data in Example 16 regarding amplification of the PRO539 gene are reasonably correlated with overexpression of the PRO539 mRNA and polypeptide such that the antibodies to the PRO539 polypeptide have utility as diagnostic tools as well. As discussed below, even if the PTO has established a reasonable doubt regarding Applicants' assertion that they are reasonably correlated, Applicants' overwhelming rebuttal evidence is more than sufficient to establish that changes in mRNA level more often than not lead to corresponding changes in protein level.

The PTO's Evidence is Not Relevant to Determining Whether a Change in mRNA Level for a Particular Gene leads to a Corresponding Change in the Level of the Encoded Protein

Applicants turn next to the second portion of their argument in support of their asserted utility – that it is well-established in the art that amplification of a gene leads to overexpression of the mRNA and protein; given Applicants' evidence of amplification of the PRO539 gene in lung and colon tumors, it is likely that the PRO539 mRNA and polypeptide are also differentially expressed; and antibodies to proteins differentially expressed in certain tumors have utility as diagnostic tools.

In response to Applicants' assertion, the PTO states that "[s]even out of eight microarray papers show discordant protein and mRNA expression data," citing Orntoft (previously submitted by Applicants); Czupalla *et al.* (Proteomics, 2005; 5:3868-3875); Kwong *et al.* (Genomics, 2005; 86:142-158); Chen *et al.* (Mol. Cell. Proteomics, 2002; 1:304-313); Conrad *et al.* (Mol. and Cell. Proteomics, 2005; 4:1284-1296); Ginestier *et al.* (Am. J. Pathology, 2002; 161:1223-1233); Anderson and Seilhamer (Electrophoresis, 1997; 18:533-37) and Washburn *et al.* (PNAS, 2003; 100:3107-3112). *Office Action* at 4-7. In addition, the PTO repeats its

previous argument that "[a]bundant art supports the absence of a necessary relationship between mRNA and protein," citing Meric, Gökman-Polar, and Pennica. *Office Action* at 7-8.

Applicants turn first to the "seven of eight" references which the PTO asserts show discordant protein and mRNA expression. As an initial matter, Applicants reiterate that Applicants' asserted utility does not require that all changes in protein levels result from changes in mRNA level. Applicants' assert that, as a general rule, gene amplification leads to overexpression of the mRNA and encoded protein. The fact that protein levels can change in the absence of gene amplification or a change in mRNA is not relevant to Applicants' asserted utility – Applicants are not trying to determine or predict mRNA levels by examining changes in protein level.

a. Czupalla et al.

The PTO states that "[t]he data of Czupalla, which addresses 117 genes, shows that it is more likely than not in this data set that there is no correlation between mRNA expression and protein expression. This supports the conclusion that mRNA expression cannot be relied upon for enablement and utility of the protein since no necessary correlation exists. *Office Action* at 4 (emphasis added).

This argument fails for two reasons. First, as stated numerous times, a "necessary" correlation is not required for utility – a reasonable correlation is all that is required. The PTO's failure to accept the proper standard in light of clear teaching from the Courts is baffling. Second, the PTO's conclusion is not supported by Czupalla, which reports on changes in protein expression in osteoclastogenesis. As the PTO notes, the authors report finding two groups of proteins: those where there is a change in mRNA with a corresponding change in protein, and those where there is a change in protein and no change in mRNA. As discussed above, and previously, Applicants are not concerned with predicting mRNA levels from changes in protein, and therefore a lack of correlation in the second group is irrelevant. As for the first group, the authors report that "the first group comprises 47 genes for which differences in mRNA expression and in abundance of the corresponding protein spots on 2-D gels were consistently detected," listing genes "which are increased on the mRNA and protein level," as well as genes "for which mRNA and protein expression are downregulated." *Czupalla* at 3873, right column. The authors conclude by stating that "[t]hus, differential expression of many genes during

osteoclastogenesis was confirmed by two complimentary techniques." *Id.* at paragraph bridging 3873-3874. Only three of the 47 genes from the first group are mentioned as having discordant mRNA and protein changes. *Id.* at 3874, right column, first paragraph. Apparently, the other 44 genes, or 94%, showing a change in mRNA had a corresponding change in protein. Therefore, rather than supporting a lack of correlation between changes in mRNA leading to changes in protein, Czupalla actually strongly supports Applicants' position.

b. Kwong et al.

The PTO states that Kwong analyzed 47 genes in colorectal cancer, and that "Only 12 of 47 genes exhibited correlated expression at a significance level less than 0.05. Surprisingly, 13 genes had a negative correlation between mRNA and protein levels." *Office Action* at 5. The PTO also relies on Kwong's statement that "correlation between protein and mRNA was also compared on a sample-by-sample basis. Of the 53 samples for which data was available, mRNA and protein levels were found to be correlated at a significance level of 0.05 in only 14 samples, while 14 mRNA and proteins were negatively correlated." *Id.* Based on Kwong, the PTO concludes that "Kwong supports the conclusion that it is more likely than not that there is no correlation." *Id.*

The PTO's reliance on Kwong is misplaced because neither portion of Kwong is contrary to Applicants' position that changes in mRNA expression levels lead to changes in protein expression levels. In the first portion of the paper quoted by the PTO, the authors report that of 47 genes examined at the mRNA and protein level, only 12 exhibited a significant correlation between the two measures. This study is not applicable to Applicants' assertion because the authors were not comparing changes in mRNA between normal and cancerous tissue against protein levels in normal and cancer tissue. The correlation relied on by the PTO is simply a plot of RNA level versus protein level for all of the samples where both RNA and protein was detected – the authors were not examining differential mRNA expression between normal and tumor tissue to see if there was a corresponding change in protein level. Of the 47 genes examined, only two are identified by the authors as having differentially expressed mRNA between the normal and tumor samples (PCNA and CKB; see Table 9, and compare to Tables 2, 3, 6 and 7, and text on page 146, right column, first paragraph), and both of these genes show a correlation (r=0.567 and 0.545) between mRNA and protein. As for the other 45 genes in Table

9, there is no indication that mRNA levels are differentially expressed between normal and tumor samples, and therefore they say nothing about whether changes in mRNA lead to changes in protein expression.

That the authors were not looking at differentially expressed genes is clear from the fact that under the heading "Differential expression of mRNA and proteins," the authors discuss the mRNA and proteins that were differentially expressed between tumor and normal tissue. The authors state that "cross-validation between RNA and protein expression levels on a gene-by-gene basis was not performed since the overlap between the identified proteins and the probes on the cDNA microarray was very limited." *Kwong* at 146, right column, second paragraph (emphasis added). Because an analysis of the differentially expressed mRNAs and proteins was not conducted, Kwong does not address the issue of whether changes in mRNA lead to changes in protein.

As for the second portion of Kwong, where the authors looked at mRNA and protein levels on a sample-by-sample basis, this is also irrelevant to the issue of whether a change in mRNA level leads to a change in protein level. In a sample-by-sample comparison, the authors are looking for a correlation between the level of mRNA and corresponding protein by plotting a single measurement of mRNA level vs. protein level for the 47 different genes in a single sample. The only way that such a plot would result in a significant correlation is if there exists a ratio between mRNA levels and protein levels that is the same across all genes, *i.e.*, that for every X copies of an mRNA, there are Y copies of the encoded protein, such that the ratio of X:Y is constant across all genes. The data from Kwong indicates that only 14 of 53 samples showed a positive correlation in this analysis. All this proves is that in most samples examined, the ratio of mRNA:protein level varies for different genes, *i.e.* no common ratio exists. This does not mean that increasing or decreasing mRNA levels for a particular gene will not result in an increase or decrease in protein level.

Applicants' asserted utility does not require knowledge of, or even the existence of, a common ratio between mRNA levels and protein levels across different genes. Nor do Applicants' assertions require calculation of protein levels based on measured mRNA levels. Applicants are not relying on a single measure of mRNA for a particular gene and then attempting to calculate protein levels based on a common global ratio between mRNA and

protein levels. Instead, Applicants are relying on differential mRNA expression, where mRNA levels are measured in two different conditions, i.e. tumor and normal. Applicants assert that a change in mRNA expression level for a particular gene typically leads to a corresponding change in the expression level of the encoded protein. The Kwong reference is applicable to only a completely unrelated issue – whether a single measure of mRNA levels can be used to predict protein levels – and therefore, this reference has no bearing on Applicants' assertions.

c. Chen et al.

The next reference relied on by the PTO is by Chen *et al.* The PTO quotes Chen as stating: "By comparing the mRNA and protein expression levels within the same tumor samples, we found that 17% (28/165) of the protein spots (21/98 genes) show a statistically significant correlation between mRNA and protein." *Office Action* at 5. The PTO also relies on Chen's statement that: "The majority of protein isoforms, however, did not correlate with mRNA levels and thus their expression is regulated by other mechanisms. We also observed a subset of proteins that demonstrated a negative correlation with the mRNA expression values." *Id.*

As an initial matter, it is important to note that a portion of Chen apparently not relied on by the PTO is irrelevant to Appellants' assertion that changes in the level of mRNA lead to changes in the level of the encoded polypeptide. In one experiment similar to that of Kwong, Chen examined the relationship between mRNA and the corresponding protein abundance by calculating the average mRNA and protein level of all the samples for each gene or protein, and then looked for a correlation across different genes. This is similar to the sample-by-sample comparison done by Kwong, only the data were averaged across all samples. Based on these data, Chen reported that "no significant correlation between mRNA and protein expression was found ($r = -0.025$) if the average levels of mRNA or protein among all samples were applied across the 165 protein spots (98 genes)." *Chen* at Abstract. This measurement of a correlation across different genes is not relevant to Appellants' asserted utility for the same reasons discussed above with respect to the Kwong *et al.* reference – it merely shows that in the samples tested, there is no common or global ratio of mRNA:protein that applies across all genes.

Chen also looked at the level of mRNA of 98 individual genes and their corresponding proteins by plotting the data from across the samples. Chen reports that 17% (28 of 165) of the protein spots, or 21.4% (21 of 98) of the genes, showed a statistically significant correlation

between protein and mRNA expression. *Chen* at Abstract. It is these results that the PTO relies on for support.

However, read in its entirety, Chen provides scant evidence to counter Applicants' assertions because Chen provides little insight into the relationship between changes in mRNA levels and changes in the corresponding protein levels for mRNA that is differentially expressed in tumor cells relative to normal cells.

Applicants have asserted that changes in mRNA levels will correspond with measurable changes in polypeptide expression. Like Kwong, Chen did not examine differential mRNA expression between normal and cancer samples, and then attempt to determine if the differential mRNA expression results in differential protein expression. Stated differently, if there is no substantial change in mRNA levels for a particular gene, one cannot measure a correlation between changes in mRNA and changes in the encoded protein for that gene. Therefore, one must know if the individual genes studied by Chen were differentially expressed to know if the observed lack of correlation has any relevance to Applicants' assertions of a general correlation between changes in mRNA and protein.

Importantly, unlike Applicants, Chen did not examine differences in mRNA between tumor and normal tissue where one would expect to find substantial changes in the level of mRNA for certain genes. Instead, Chen merely selected proteins whose identity could be determined regardless of any changes in expression level. *Chen* at 306, right column. Therefore, it is not known if there was any substantial difference in mRNA levels for the various studied genes across samples – in short, with the exception of the genes in Figures 2A-2C, where a correlation is observed, it is not known if the genes examined were differentially expressed. Also of significance for Applicants' asserted utility is the fact that Chen did not attempt to examine any differential expression between the cancerous lung samples and the non-cancerous lung samples – Chen did not distinguish between cancer and normal samples in their analysis. In the absence of substantial differential expression, no correlation would be observed. As discussed above with respect to Kwong, because it is not known if there was a change in the level of mRNA for the genes studied by Chen, *i.e.* whether they were differentially expressed, the lack of an observed correlation cannot be used to counter Applicants' assertion.

In sum, the only data reported by Chen which shows substantial changes in the expression of mRNA, Figures 2A-C, supports Applicants' assertion that substantial changes in mRNA levels will correspond to substantial changes in polypeptide expression since a correlation was observed. As for the lack of an observed correlation between mRNA levels and protein levels for other genes reported by Chen, no conclusion can be drawn since there is no indication the genes are differentially expressed. Thus, Chen's results do not refute Applicants' position. If anything, Chen supports Applicants' position that a significant correlation between changes in mRNA and protein levels exists for changes in mRNA levels.

d. Conrad et al.

The PTO relies on Conrad, asserting that comparing the abundance of protein to nucleic acid microarray for proteins, Conrad reported "There is little correlation between RNA and protein abundance identified and predicted by cICAT." *Office Action* at 6-7.

The PTO's reliance on Conrad is misplaced for the same reason that the second portion of Kwong is not relevant to Applicants' assertion. Conrad reports on a combined proteome and microarray investigation of pre-osteoblasts stimulated with inorganic phosphate, examining the correlation between mRNA and protein levels across ~1900 different genes. However, like Kwong, the authors of Conrad plotted a single time point for mRNA against a single time point for protein expression, looking for a correlation across the ~1900 genes. The only way this analysis will result in a significant correlation is if the ratio of mRNA:protein is constant across all ~1900 genes. Regardless of whether genes share a common mRNA:protein ratio or not is irrelevant because this type of experiment cannot address whether differential mRNA expression for a single gene generally results in differential protein expression for the corresponding protein – the two issues are independent of each other.

e. Ginestier et al.

The PTO also relies on Ginestier, stating that table 4 teaches that only 5 of 15 genes showed concordance, and that the authors report that "For a category of molecules we found important differences between RNA and protein expression levels." *Office Action* at 6.

The Ginestier reference is difficult to interpret given the way the mRNA and protein samples were analyzed. The authors examined the level of mRNA and protein expression for 15 genes in 55 breast tumor samples. The authors then classified the protein and mRNA expression

level for a particular gene in each sample as being either a class 1 (low), class 2 (mid), or class 3 (high). A contingency table analysis was then used to analyze the relationship between protein and mRNA expression for each gene. The authors report that only 5 of the 15 genes showed a significant relationship.

However, this analysis ignores the fact that in many cases, a relationship was not found because there were more samples with a "high" (class 3) protein expression level than there were samples with a "high" (class 3) mRNA level. For example, FGFR1 mRNA has 45 "low" (class 1), 10 "mid" (class 2), and 0 "high" (class 3) samples compared to FGFR1 protein which has 20 "low" (class 1), 20 "mid" (class 2), and 15 "high" (class 3) samples. *Ginestier* at Table 4. This is not contrary to Applicants' assertion, as Applicants are not arguing that "high" protein levels are always and only due to "high" mRNA expression. Applicants' assertion is that increasing mRNA levels lead to increasing protein levels, not the other way around. Thus, a lack of concordance because there are more "high" or "mid" protein samples than there are "high" or "mid" mRNA samples for a particular gene is not contrary to Applicants' assertion.

In addition, the assignment of the protein and mRNA expression levels to class 1, 2, or 3 was arbitrary, and influences whether a relationship is seen or not (for example, for some molecules, protein expression characterized as "strong" (S) in Table 3 is classified as class 3 (high) in Table 4 (e.g., BCL2) while for other molecules "strong" (S) protein expression is classified as class 2 (med) in Table 4 (e.g., Ki67)). These flaws, combined with the fact that Conrad was not looking at differential mRNA expression (*i.e.* a change in mRNA between condition X and condition Y), make Conrad of little value in assessing whether one of skill in the art would believe Applicants' assertions regarding differential mRNA expression leading to differential protein expression.

f. Anderson and Seilhamer

The PTO argues that: "Anderson et al shows that for 19 proteins that were compared between 20 gel electrophoresis and mRNA analysis 'the correlation coefficient obtained over this set of data was 0.48. This number is intriguingly close to the middle position between a perfect correlation (1.0) and no correlation whatever (0.0).' In fact, the correlation is slightlycloser to showing that there is no correlation whatsoever between protein and mRNA data." *Office Action* at 6.

Like the second portion of Kwong, part of Chen, and Conrad, the Anderson paper is irrelevant because the authors examined the correlation between mRNA and protein across different genes – this is a search for a common global ratio of mRNA:protein. Applicants are asserting that if gene X is increased in cancer tissue relative to normal tissue, then protein X will be increased in cancer tissue relative to normal tissue. The truth of that assertion does not depend on a common global ratio of mRNA:protein across different genes.

To exemplify the difference between these references and Applicants' asserted utilities, Applicants offer the following illustration and analogy with the understanding that like all illustrations and analogies, they are not perfect and therefore do not represent any admissions or binding statements regarding Applicants' disclosure or invention.

Kwong, Chen, Conrad and Anderson discuss whether there is a correlation between a single measure of mRNA and protein level globally, *i.e.* across different genes at a given time. This is equivalent to conducting a hypothetical Experiment 1, where a particular cell type has 100 copies of mRNA for gene X, 200 copies of mRNA for gene Y, and 400 copies of mRNA for gene Z. If there is a common global ratio of mRNA:protein such that there is a correlation between mRNA levels and protein levels across genes, the relative amount of proteins X:Y:Z would be approximately 1:2:4 since this is the relative amount of their respective mRNAs. This is essentially what the cited references examined.

In contrast, Applicants are relying on a correlation between changes in mRNA level for a particular gene leading to a corresponding change in the level of the encoded protein when comparing tissues at two different times or conditions. For example, in hypothetical Experiment 2, if gene X has 100 copies of mRNA per cell in condition A (*e.g.* normal), and 200 copies of mRNA for gene X in condition B (*e.g.* tumor), the amount of protein X in condition A would be smaller than the amount of protein X in condition B, for example, having a ratio of 1:2, such that there is a correlation between the difference in the level of mRNA and the difference in the level of protein for a particular gene.

By relying on Kwong, Chen, Conrad and Anderson, the PTO is apparently arguing that because there is no correlation between levels of mRNA and protein across genes in a particular sample, as illustrated by Experiment 1, one of skill in the art would not expect an increase or decrease in the amount of mRNA for a particular gene to result in a corresponding change in the

amount of the encoded protein, as illustrated in Experiment 2. This is simply wrong – there does not need to be a global ratio of mRNA:protein across genes for there to be a correlation in changes of mRNA and protein for a particular gene.

The findings of Anderson that different amounts of mRNA result in different amounts of protein when looking across genes in a sample is analogous to finding that on one gallon of gas, a hybrid car can travel 50 miles, an SUV can travel 20, but a large truck can only travel 5 miles. If gas were plotted against miles for a variety of automobiles, there would be no correlation between gas and miles. That is to say, there are many things which affect the fuel efficiency of an automobile. Based on these observations, one could conclude that given the lack of a global ratio of gas to miles, and the resulting lack of correlation between the amount of gas in an automobile and the distance it travels, one cannot predict how far an automobile will travel based on a single measure of the amount of gas in the tank – one could say conclude that it is more likely than not that there is no correlation between gas and miles.

Even if true, the data and conclusions of Kwong, Chen, Conrad and Anderson are irrelevant to Applicants' assertions. Regardless of the fact that there are numerous levels of control of protein expression, Applicants assert that, generally speaking, increasing the amount of mRNA for a particular gene will result in a corresponding increase in the amount of the encoded protein. This is analogous to increasing or decreasing the amount of gas in an automobile – it will travel farther if you add more gas, and not as far with less. The fact that there are many things which affect fuel efficiency and therefore you cannot predict how far an automobile will travel without knowing if it is a hybrid or a large truck is irrelevant – both a hybrid and a truck travel farther on more gas, and not as far on less. A lack of correlation between gas and miles when examined across different automobiles does not mean that generally speaking, increasing the amount of gas means the automobile will travel further – they are independent and unrelated questions.

Applicants emphasize, and the PTO will recognize, that these are simplified illustrations to demonstrate the difference between the two issues being examined. However, these illustrations make clear that even if there is no correlation in the first experiment looking at levels of mRNA and protein across genes in a sample, there can still be a correlation between changes in mRNA and protein for a particular gene as examined in the second experiment.

g. Washburn et al.

The final reference cited by the PTO is Washburn, which the PTO asserts found a correlation of 0.45 when examining 678 loci. *Office Action* at 6. The PTO concludes that this is closer to the absence of a correlation than to a positive correlation. *Id.*

The authors of Washburn conducted an experiment which more directly addresses Applicants' assertion since they looked at the relationship between changes in mRNA and protein expression in yeast grown in minimal and rich media. The authors plotted the log of the ratio of mRNA expression in minimal and rich media against the log of the ratio of protein expression in minimal and rich media. The authors report a weak (0.45) correlation.

However, the authors note that "[a] majority of the data points deviating from the perfect positive correlation line shown fall on the y axis indicating that more loci had altered protein expression and unchanged mRNA expression than loci having altered mRNA expression and unchanged protein expression." *Washburn* at 3109 column 1. In fact, looking at Figure 2, if the data points for loci where the mRNA levels did not change by at least two-fold are eliminated (*i.e.*, values between -1 and 1), it appears that there would be an excellent correlation between the changes in mRNA and the changes in protein. As Applicants' have previously stated, changes in protein level without changes in mRNA are not relevant to Applicants' assertion. The question is whether an increase in mRNA generally results in a change in protein. Based on the data in Washburn, the answer appears to be "yes." Thus, Washburn is not contrary to Applicants' assertion, but rather supports it.

h. Conclusion – the references cited are not contrary to Applicants' assertion

The PTO concludes its discussion of the above references by stating:

> Combining the data from Czupalla, Kwong, Orntoft, Chen and Ginestier, they analyzed 384 genes in total. There was a correlation between the RNA and protein levels for 131 of these genes (with 39 of them being the highly correlated Omtoft paper). This results in a final correlation of 34%, which means that it is more likely than not that there is no correlation between RNA and protein levels. So not only is there no necessary connection between the level of protein in a cell and the amount of mRNA, but there is also no necessary correlation between the amount of DNA in a cell and the amount of mRNA. Therefore, any evidence by Applicant showing overexpression of one component does not provide utility for the protein itself. *Office Action* at 6-7 (emphasis added).

This conclusion is flawed because, for the reasons discussed above, the PTO is relying on references and data that simply are not relevant to Applicants' assertion, and ignoring the numerous references cited by Applicants in their previous response. In addition, none of the references cited examined the relationship between DNA and mRNA, other than Orntoft, which found a strong correlation, and therefore there is no support in these references for the PTO's conclusion that there is no correlation between DNA and RNA. Finally, Applicants again remind the PTO that the standard is not a "necessary" correlation, but merely a "reasonable" one.

When examining a reference to determine whether it supports Applicants' assertions, one must always keep in mind what question is being asked. The question is not whether more copies of mRNA X than mRNA Y in a particular sample leads to more protein X than protein Y. The answer to that question is not relevant. The question at hand is whether more copies of mRNA X in condition B as compared to condition A results in more protein X in condition B as compared to condition A. When each of the references cited by the PTO is actually examined closely with the proper question in mind, rather than simply looking for statements regarding "correlations," it is apparent that none of the references cited by the PTO are contrary to Applicants' assertion. The references are either completely irrelevant, (parts of Kwong, parts of Chen, Conrad, and Anderson), do not provide enough information to make an assessment of their relevance, (parts of Kwong, parts of Chen, and Ginestier), or actually support Applicants' position (Orntoft, Czupalla, and Washburn). Far from establishing "that it is more likely than not that there is no correlation between RNA and protein levels," the cited references on whole support Applicant's position.

i. Meric, Gökman-Polar, and Pennica

In addition to the eight new references discussed above, the PTO also repeats its previous argument that "[a]bundant art supports the absence of a necessary relationship between mRNA and protein," citing Meric, Gökman-Polar, and Pennica. *Office Action* at 7-8 (emphasis added).

Applicants have previously discussed at length why the Meric, Gökman-Polar and Pennica references do not support the PTO's position. Briefly stated, Applicants argue that Meric supports Applicants' assertion that generally, changes in mRNA lead to a corresponding change in the level of the encoded protein – that is why examining differences between tumor and normal tissue at the mRNA level is a "fundamental principle" of molecular cancer

therapeutics. Likewise, Meric teaches that mRNA overexpression can be attributed to gene amplification. Gökman-Polar reports only that increased protein levels were not accompanied by increased mRNA levels – this is not contrary to Applicants' assertion that gene amplification leads to overexpression of an mRNA which leads to overexpression of the corresponding protein. In addition, Gökman-Polar reports a positive correlation between changes in mRNA level and changes in protein level for five of six samples tested. Finally, Pennica, the only reference which looked at a correlation between gene amplification and gene expression, reports one gene where there was a strong correlation between the two, and one possible example where there was a lack of positive correlation. This evidence is at best inconclusive, with at least half the genes showing a correlation between gene amplification and mRNA overexpression.

While these references possibly establish that there is no "necessary" correlation between gene amplification and overexpression of mRNA and protein, Applicants are not required to establish the asserted utility beyond a reasonable doubt or to a statistical certainty. Thus, even assuming that the PTO has proved that there is no "necessary" correlation, this does not mean that it has met its initial burden of establishing that it is "more likely than not" that a skilled artisan would doubt the asserted utility. Given that two of the cited references actually support Applicants' position, Applicants assert that the PTO's continued reliance on these references is misplaced.

Conclusion – the PTO's evidence is not sufficient to provide a basis for one of skill in the art to doubt Applicants' asserted utility

Applicants have shown that the references relied on by the PTO are either irrelevant, not contrary to Applicants' assertions, or actually support Applicants' position, not the PTO's. Taken together, the PTO's arguments are not sufficient to satisfy the burden to "provide[] evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility." *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995).

Applicants' Evidence Establishes that a Amplification of a Gene Leads to Overexpression of the Corresponding mRNA

In support of the assertion that gene amplification results in overexpression of the corresponding mRNA, Applicants previously submitted numerous references and an expert

declaration. How these references support Applicants' assertions are of record and will not be repeated here. While Applicants have previously acknowledged that the correlation between gene amplification and mRNA overexpression is not a "necessary" or exact one, Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the general principle that there is a correlation between gene amplification and mRNA overexpression does not provide a proper basis for rejecting Applicants' asserted utility.

Applicants' Evidence Establishes that a Change in mRNA Level for a Particular Gene lead to Corresponding Change in the Level of the Encoded Protein

In support of the assertion that changes in mRNA, *e.g.* an increase, are positively correlated to changes in protein levels, Applicants previously submitted several expert declarations, excerpts from textbooks, and numerous references where the authors conducted experiments that are relevant to evaluating Applicants' assertion – unlike most of the references cited by the PTO. The details of the teachings of these declarations and references, and how they support Applicants' asserted utility, are of record and will not be repeated here.

Applicants submit herewith a copy of a declaration by Randy Scott, Ph.D. (attached as Exhibit 1). Dr. Scott is an independent expert in the field of molecular diagnostics, with over 15 years experience. He is the author of over 40 scientific publications in the fields of protein biology, gene discovery, and cancer, and is an inventor on several issued patents. His curriculum vitae is attached to the declaration. In paragraph 10 of his declaration, Dr. Scott states:

> One reason for the success and wide-spread use of the DNA microarray technique, which has led to the emergence of a new industry, is that generally there is a good correlation between mRNA levels determined by microarray analysis and expression levels of the translated protein. Although there are some exceptions on an individual gene basis, it has been a consensus in the scientific community that elevated mRNA levels are good predictors of increased abundance of the corresponding translated proteins in a particular tissue. Therefore, diagnostic markers and drug candidates can be readily and efficiently screened and identified using this technique, without the need to directly measure individual protein expression levels. *Scott Declaration* at ¶10 (emphasis added).

Applicants submit the opinion of yet another expert in the field that changes in mRNA level for a particular protein in a given tissue generally lead to a corresponding change in the level of the encoded protein. Importantly, Dr. Scott also states that, contrary to the contentions of the PTO, diagnostic markers can be identified "without the need to directly measure individual protein expression levels." This opinion is supported by Dr. Scott's extensive experience in the field, as well as the fact that an entire industry has developed around technology to assess differential mRNA expression. As stated previously, there would be little reason to study changes in mRNA expression levels if those changes did not result in corresponding changes in the encoded protein levels.

The case law has clearly established that in considering affidavit evidence, the PTO must consider all of the evidence of record anew. *See in re Rinehart,* 531 F.2d 1084, 189 USPQ 143 (C.C.P.A. 1976); *In re Piasecki,* 745 F.2d. 1015, 226 USPQ 881 (Fed. Cir. 1985). "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument." *In re Alton,* 37 U.S.P.Q.2d 1578, 1584 (Fed. Cir. 1996), *quoting In re Oetiker,* 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992). Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an examiner." *Id.* at 1583. Applicants also respectfully draw the PTO's attention to the Utility Examination Guidelines which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered." *66 Fed. Reg. 1098, Part IIB (2001).*

In summary, Applicants have submitted herewith an additional expert Declaration in addition to the declarations and over 115 references already of record, which support Applicants' asserted utility, either directly or indirectly. This evidence supports the assertion that in general, a change in mRNA expression level for a particular gene leads to a corresponding change in the level of expression of the encoded protein. As Applicants have previously acknowledged, the correlation between changes in mRNA level and protein level is not exact, and there are exceptions. However, Applicants remind the PTO that the asserted utility does not have to be

established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for rejecting Applicants' asserted utility. Applicants submit that considering the evidence as a whole, with the overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true." *Id.*

In conclusion, Applicants submit that they have offered sufficient evidence to establish that it is more likely than not that one of skill in the art would believe that because the PRO539 gene is amplified in lung and colon cancer tissue, the PRO539 mRNA and polypeptide will likewise be differentially expressed. This differential expression of the PRO539 polypeptide makes the claimed antibodies useful as diagnostic tools for cancer, particularly lung or colon cancer.

The PTO's Arguments in Response to Applicants' Evidence of Record are Contrary to the PTO's Own Guidelines

In Applicants' most recent response, Applicants submitted over 100 additional references in support of their assertion that gene amplification leads to overexpression of the mRNA for the gene, which is positively correlated to changes in the corresponding protein level. In response, the PTO argues:

> None of these argued references, other than Orntoft, represents the sort of global comparison of many (as many as 2501) different proteins to their corresponding mRNAs that was performed by the microarray comparison papers cited in the action above. In fact, Applicant selectively chooses art to support one position, because Applicant has several of the microarray papers such as Anderson and Chen listed in the IDS, but fails to discuss the teachings of these papers in the arguments. *Office Action* at 21.

Applicants note that there is no requirement that only "global comparisons" be cited as evidence to support Applicants' position – Applicants are free to rely on any reference which supports their position, and the PTO is obligated to consider it. As explained above, most of the "global comparisons" relied on by the PTO are irrelevant because they do not examine whether changes in mRNA lead to changes in protein level. As for the PTO's accusation that Applicants' are selectively discussing art to support their position, Applicants have chosen to discuss in detail

the references they view as most relevant to addressing their asserted utility. References such as Anderson and Chen are not relevant for the reasons discussed above, and therefore Applicants' have not discussed them.

As examples, the PTO cites two additional references which Applicants' disclosed to the PTO but did not discuss. The PTO states that these references "which perform global comparisons of mRNA and protein, are concordant with the rejection, not Applicant's position." *Office Action* at 22.

The first reference the PTO discusses is the Waghray reference. The PTO argues that Waghray "supports the conclusion that there is a lack of concordant change" between mRNA and protein. *Office Action* at 22-23.

Waghray *et al.* looked at transcriptosomal and proteosomal changes in an androgen-sensitive prostate cancer cell line after the cells were treated with dihydrotestosterone (DHT). Out of 16,570 genes, the authors found 351 transcripts that were differentially expressed in the stimulated cells. The authors also identified 44 proteins, out of 1031 spots on protein gels, that were upregulated or downregulated in stimulated cells. Hence, Waghray *et al.* found that over 4% (44/1031) of the proteins isolated from the cells were differentially expressed while only 2% (352/16,570) of the transcripts were differentially expressed. Waghray *et al.* found that corresponding SAGE (sequence analysis) data were available for a number of the proteins identified as differentially expressed and stated that "[i]nterestingly, for most of the proteins identified, there was no appreciable concordant change at the RNA level." *Office Action* at 22. The PTO concludes from this statement that the data presented by Waghray *et al.* support its argument against a correlation between mRNA levels and protein levels.

However, further analysis of the data collected in these experiments shows that such a conclusion cannot be drawn from the data. The experiments of Waghray *et al.* that produced the data shown in Table 4 involve hormonally stimulating cells for 24 hours; determining mRNA levels in the cells; and, 48 hours after determining mRNA levels, determining protein levels, for specific mRNA/protein product pairs. The authors measured mRNA levels twice, before stimulating with DHT and after stimulating with DHT for 24 hours (24 hours post-treatment). They also measured protein concentrations twice, before stimulating with DHT and at 72 hours post-treatment. The second measurement of protein levels therefore occurred 48 hours after

DHT was removed from the culture media. Thus, the experiment involved creating a dynamic and changing environment for cells and the measurement of the effects of this changing environment at only one timepoint. Additionally, the timepoints used for measuring the effects on mRNA levels and protein levels were 48 hours apart.

Examining the two timepoints for particular genes, the authors stated that there was not appreciable concordant change at the RNA level for most of the proteins whose concentrations were affected by DHT treatment. However, the differential expression of mRNA at 24 hours and of protein at 72 hours does not reveal the complete picture of the effects of DHT treatment on the cells. The authors noted that the dynamic conditions of the experiments created fluctuating levels of both mRNA and protein over time (pg. 1337). They decided to examine the kinetics of mRNA and protein levels for two proteins affected by DHT treatment, PSA and clusterin (Fig. 1C on pg. 1334). PSA is known to be an androgen-regulated gene and the authors had been surprised to see only a 1.7 fold induction of PSA transcripts by DHT treatment at the 24 hour timepoint. But through the kinetic experiment, they saw that induction of PSA began between 4 and 6 hours post-treatment and they detected a 5 to 10 fold induction of PSA at 6 to 8 hours post-treatment. PSA mRNA levels subsequently declined, so that by the time samples were taken for SAGE analysis at 24 hours post-treatment, only a 1.7 fold induction was seen. The results of the clusterin kinetic experiment show an even greater effect of DHT treatment on induction and greater fluctuation ranges. Clusterin mRNA induction began sooner than PSA induction (only 0.5 to 1 hour post-treatment), declined between 6-12 hours post-treatment, and at the 24 hour timepoint clusterin mRNA levels had declined to a lower level than the untreated control cells. Thus, while clusterin mRNA was initially induced to much higher than steady-state levels by DHT treatment, by the time the researchers quantified the levels of clusterin mRNA with SAGE at the 24 hour timepoint, clusterin mRNA levels had fallen *below* the levels measured pre-treatment.

Due the dynamic nature of these stimulation experiments, it is clear that the observed effect of DHT treatment on the mRNA level of an affected gene will depend on *when* the observation is made. For example, with clusterin, one could observe a large induction of transcription (1-6 hours post-treatment), no change in mRNA levels (some point between 12 and 24 hours post-treatment), or a reduction *below untreated levels* of mRNA (24 hours post-

treatment), all depending on the particular timepoint chosen for the collection of an RNA sample from treated cells. Because of these fluctuations of mRNA levels over time, the data from Table 4 have no relevance to the relationship between steady-state levels of mRNA and protein for a particular gene and cannot inform us as to the general relationship between mRNA levels and protein levels. This is especially true since the authors did not perform kinetic experiments on proteins affected by DHT treatment; it is unknown whether reduced levels of expression seen for some proteins in the table represent a persistent suppression of protein expression over a 72 hour period or merely a reduced level at just the 72 hour timepoint. Thus, the data from Table 4, upon which the authors base their observation about the concordance of mRNA and protein levels, actually provide no insight into the relationship between mRNA levels and protein levels in a dynamic experiment with stimulated cells, let alone for cells in a steady-state environment.

The PTO has cited the observations of Waghray *et al.* regarding their experiments on stimulated cells in support of its argument that mRNA levels are not necessarily predictive of protein levels, even when there are changes in the mRNA level. But because of the differences in transcript and protein detection efficiency and the dynamic nature of the stimulation experiments, no correlations between transcript and protein levels can be accurately drawn from the data presented. The conclusions of the authors have no relevance to and do not support the PTO's argument.

The second reference the PTO cites is an article by Gygi *et al.*, quoting Gygi as stating "In conclusion, this study examined the relationship between yeast protein and message levels and revealed that transcript levels provided little predictive value with respect to the extent of protein expression." *Office Action* at 23.

Gygi is another example of a reference which is examining the level of mRNA and protein in a sample across different genes. As discussed at length above with reference to the Kwong, Chen, Conrad and Anderson references, a search for a common global ratio between mRNA and protein which applies to all genes in a sample is simply not relevant to Applicants' assertions. Therefore, Gygi offers no support for the PTO's rejection of Applicants' assertions, and there was no reason for Applicants' to discuss Gygi or the Waghray references.

Finally, the PTO addresses three of the references cited by Applicants: Gromova, Aust, and Kuo. The PTO quotes Gromova as stating that based on Anderson and Seilhamer, it was

important to determine the levels of the molecules being studied at both the mRNA and protein level. *Office Action* at 23. This quote is not contrary to Applicants' assertion, because Anderson and Seilhamer is not relevant since they examined the relationship between mRNA and protein levels across different genes in the same sample, as discussed in detail above. In addition, when the molecule was examined at the mRNA and protein level, the authors report that "[o]verall, the results showed a good correlation between protein abundancy and mRNA levels." *Gromova* at Abstraact. Thus, the portion of the reference cited by the PTO is not contrary to Applicants' position, and the finding of a correlation supports it.

The second reference mentioned by the PTO is Aust *et al.* The PTO quotes Aust as stating that "the discrepancy between elevated TIMP-1 mRNA levels of thyrocytes and the extremely low TIMP-1 protein secretion by these cells is difficult to explain. Post transcriptional regulatory events may be responsible for this confounding result." *Office Action* at 24 (emphasis added). This statement actually supports Applicants' assertion that generally, increased mRNA leads to increased protein because the authors refer to an example where this apparently did not hold true to be "difficult to explain" and a "confounding result." In addition, Aust reports that when they examined MMP-1 mRNA and protein levels, they found "a strong correlation between levels of MMP-1 mRNA and protein ($r = 0.99$, $p < .0001$)." *Aust* at Abstract.

Finally, the PTO quotes a single line from Kuo *et al.*: "Comparison of the gene and protein expression profiles showed that there was a discordance between mRNA and protein levels." *Office Action* at 24. In Kuo, the authors used microarray analysis combined with proteomic analysis using two-dimensional gel electrophoresis to examine changes in gene expression in leukemia cell lines. The authors report that "[c]omparison of microarray and proteomic expression profiles showed poor correlation. Use of more reliable and sensitive analyses, such as reverse transcriptase polymerase chain reaction [RT-PCR], Western blotting and functional assays, on several genes and proteins, nonetheless, confirmed that there is indeed good correlation between mRNA and protein expression." *Kuo et al.* at Abstract (emphasis added). Thus, Kuo supports Applicants' position.

The PTO concludes its discussion of Applicants' evidence by stating that "including all of the global comparison papers, 9 out of 10 comparison papers fail to support Applicant's position.

9 out of 10 papers which analyzed multiple genes found discordant results between mRNA and protein." *Office Action* at 24.

Applicants submit that this does not represent full consideration of the totality of the record in fairly evaluating the utility of the claimed antibodies. As provided in the M.P.E.P.:

> It is essential for Office personnel to recognize, fully consider and respond to each substantive element of any response to a rejection based on lack of utility. Only where the totality of the record continues to show that the asserted utility is not specific, substantial, and credible should a rejection based on lack of utility be maintained. *M.P.E.P.* § 2107

Applicants submit that the issue of whether changes in the level of mRNA for a particular gene lead to corresponding changes in the level of the encoded protein requires a determination based on the totality of the record, not just select papers that examined "global comparisons." The totality of the record as it stands is clear: one skilled in the art would reasonably believe that changes in mRNA levels typically lead to corresponding changes in the levels of the encoded protein. There is no basis in PTO policy or the standards set by the courts for the PTO to ignore evidence submitted by Applicants because the references examine only one or a few genes. Nor is there a basis for the PTO to make an adverse conclusion on the utility of claims based on less than the totality of the evidence. Accordingly, Applicants respectfully request that the PTO consider the totality of the references of Exhibits 1-7 and 10-19 in particular, as well as Exhibits 20-26, submitted in Applicants' previous response. Applicants submit that, contrary to the PTO's holding, given that the "global comparison" papers cited by the PTO do not support a rejection of Applicants' asserted utility for the reasons discussed above, the totality of the record shows that changes in the level of mRNA for a particular gene generally lead to corresponding changes in the level of the encoded protein, and the PTO's holding otherwise does not represent a fair and full consideration of the totality of the record, as required.

Substantial Utility - Conclusion

In conclusion, Applicants submit that they have offered sufficient evidence to establish that it is more likely than not that one of skill in the art would believe that because the PRO539 gene is amplified in lung and colon tumors, the PRO539 mRNA and polypeptide will be overexpressed in lung and colon tumors. This overexpression of the PRO539 polypeptide makes

the claimed antibodies useful as diagnostic tools for cancer, particularly lung and colon cancer. Applicants submit that considering the evidence as a whole, with the overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true." *Id.*

Specific Utility

The Asserted Substantial Utilities are Specific to the Claimed Antibodies

The PTO argues that even if substantial utility were found, there is no specific utility given for antibodies to the PRO539 protein, since antibodies to the protein, as distinguished from the nucleic acid, have not been associated with any disease, condition, or any other specific feature.

Specific utility is defined as utility which is "specific to the subject matter claimed," in contrast to "a general utility that would be applicable to the broad class of the invention." M.P.E.P. § 2107.01, part I (2004). Applicants submit that the evidence of amplification and overexpression of PRO539 nucleic acids in certain types of cancer cells along with the declarations and references discussed above provide a specific utility for the claimed antibodies. As stated above, Applicants have established a reasonable correlation between gene amplification, gene overexpression, and protein overexpression. This makes antibodies to the PRO539 protein useful in diagnosing lung and colon cancer. This is not a general utility that would apply to the broad class of antibodies.

Utility – Conclusion

Applicants remind the PTO that the evidence supporting utility does not need to be direct evidence, nor does it need to provide a "necessary" or an exact correlation between the submitted evidence and the asserted utility. Instead, evidence which is "reasonably" correlated with the asserted utility is sufficient. *See Fujikawa v. Wattanasin*, 93 F.3d 1559, 1565, 39 U.S.P.Q. 2d 1895 (Fed. Cir. 1996) ("a 'rigorous correlation' need not be shown in order to establish practical utility; 'reasonable correlation' suffices"); *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 U.S.P.Q. 739 (Fed. Cir. 1985) (same); *Nelson v. Bowler*, 626 F.2d 853, 857, 206 U.S.P.Q. 881 (C.C.P.A. 1980) (same). In addition, utility need only be shown to be "more likely than not true," not to a

statistical certainty. *M.P.E.P.* at § 2107.02, part VII (2004). Considering the evidence as a whole in light of the relevant standards for establishing utility, Applicants have established at least one specific, substantial, and credible utility. In view of the above, Applicants respectfully request that the PTO reconsider and withdraw the utility rejection under 35 U.S.C. §101.

Rejection under 35 U.S.C. §112 – Enablement

The PTO also rejects Claims 22-26 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. The PTO cites *In re Wands* and the factors set forth therein to determine the scope of enablement. The PTO's arguments are largely the same as those for the utility rejection. *Office Action* at 13-16. In particular, the PTO argues under the "unpredictability of the art and state of the art" heading that there is no "necessary" correlation between gene amplification and mRNA levels, or between mRNA and protein levels, citing the same references relied on for the utility rejection. *Office Action* at 14-15.

For the reasons of record, Applicants submit that the claimed antibodies are enabled, as one of skill in the art would know how to make and use them. Applicants submit that the evidence, declarations, references, and arguments discussed above make clear that Applicants have established that it is more likely than not that one of skill in the art would be convinced, to a reasonable probability, that the PRO539 protein is overexpressed in certain cancers, and therefore antibodies to PRO539 have utility as a diagnostic tool. To the extent that the enablement rejection is based on a lack of utility, Applicants respectfully request that the PTO reconsider and withdraw the enablement rejection under 35 U.S.C. §112.

As to the PTO's recitation of the *In re Wands* factors, Applicants note that the question of enablement regarding antibodies is the very issue that was addressed in *In re Wands*, 858 F.2d 731, 8 U.S.P.Q. 2d 1400 (Fed. Cir. 1988). In *Wands*, the CAFC held that the disclosure was sufficient to enable one of skill in the art to make monoclonal antibodies to a disclosed antigen without undue experimentation. *Id.* at 740. If the disclosure was sufficient at the time of filing of the *Wands* application in 1980, it cannot be that the art of making antibodies has become less predictable in the ensuing 25 years, and now requires undue experimentation.

In addition, Applicants submit that the specification discloses how to make and use the claimed antibodies. For example, Example 27 on page 127 of the specification specifically describes the preparation of antibodies that bind PRO polypeptides. *Specification* at 90, line 20 through 97, line 4, and 127, line 13, through 128, line 1. The specification also discloses that the claimed antibodies can be used in diagnostic assays to detect the expression of PRO539 in specific types of tissue. *Specification* at 98, lines 5-29.

Therefore, given the teaching in the specification on how to make and use the claimed antibodies to detect expression of PRO539 in specific tissues, one of skill in the art would be enabled to practice the claimed invention without undue experimentation. Thus, at least one use of antibodies to the PRO539 polypeptide is adequately enabled, which is all that is required – "if any use is enabled when multiple uses are disclosed, the application is enabling for the claimed invention." *M.P.E.P.* § 2164.01(c). In view of the above, Applicants respectfully request that the Examiner reconsider and withdraw the enablement rejection under 35 U.S.C. § 112, first paragraph.

CONCLUSION

In view of the above, Applicants respectfully maintain that claims are patentable and request that they be passed to issue. Applicants invite the Examiner to call the undersigned if any remaining issues may be resolved by telephone.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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